

KERATINOLYTIC AND KERATINOPHILIC FUNGI OF SWAMP'S SOIL AND AIR IN QENA CITY AND THEIR RESPONSE TO GARLIC EXTRACT AND ONION OIL TREATMENTS

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ABSTRACT — 48 species and one variety belonging to 25 genera were recorded from 50 soil samples and from the atmosphere of a swamp in the city of Qena using the hair baiting technique at 28° C. Twenty taxa were dermatophytes or closely related fungi. Most common and frequent species of this group were *Aphanoascus fulvescens*, *Aph. terreus* and *Aphanoascus* sp. (anamorph: *Chrysosporium tropicum*) and *Chrysosporium xerophilum*. Sixty-eight isolates were tested for their ability to grow on hair-sand medium. Most strains tested (73.5 %) had moderate growth rate. All keratinophilic fungi identified in the present investigation were sensitive to garlic extract and onion oil.

KEY WORDS: Air-spora, antifungal, garlic extract, keratinolytic and keratinophilic, onion oil.

INTRODUCTION

Soil rich in keratinic residues constitute a permanent or an occasional reservoir for dermatophytes, keratinolytic and keratinophilic fungi and is thus a potential source of infection for man and animals. Several investigations have been carried on the presence of these fungi in soil and in air in many countries all over the world (Papavassilion & Bartzokas, 1975; Alteras & Lehrer, 1977; Acosta & Roberstad, 1979; Della-France & Caretta, 1984; Chabasse, 1988). However, in Arab countries few such surveys were made (Amer *et al.*, 1975; Jana *et al.*, 1979; Youssef *et al.*, 1980, 1989; Abdel-Fattah *et al.*, 1982; Abdel-Mallek *et al.*, 1989; Abdel-Hafez *et al.*, 1989, 1991; Karam El-Din *et al.*, 1990; Moubasher *et al.*, 1990; El-Said, 1993, 1994; El-Maghraby, 1994).

The distinction between keratinophilic and keratinolytic fungi is based on the proposals of Majchrowicz & Dominik (1969) and Dominik *et al.* (1973), later on adopted by Filipello Marchisio & Luppi Mosca (1980, 1982). Keratinolytic species are defined as those fungi able to directly destroy keratin, while keratinophilic taxa are only liable to use materials naturally associated with keratin or resulting from its breakdown. The keratinolytic activity of dermatophytes using guinea-pig hair as a test substrate was measured by Yu *et al.* (1968).

Garlic (*Allium sativum* L.) extract and onion oil have a long history of reputed value and actual use for their medicinal, antimicrobial and pesticidal properties (Amon-

kar & Banerji, 1971; Shekhawat & Prasada, 1971; Fliermans, 1973; Appleton & Tansey, 1975; Tansey & Appleton, 1975; Moore & Atkins, 1977; Lewis *et al.*, 1977; Deshmukh, 1984; Yoshida *et al.*, 1987; Gherbawy, 1989; Singh *et al.*, 1990; Zohari *et al.*, 1992).

The present investigation aimed to study composition and frequency of occurrence of keratinophilic fungi in swamp's soil and air in the city of Qena and their keratinolytic activity. Also, a preliminary study on the antifungal effect of garlic extract and onion oil on isolated keratinophilic taxa was conducted.

MATERIALS AND METHODS

Fifty soil samples were collected from different swamp localities in Qena, according to the method described by Johson *et al.* (1959). Soil samples were analysed chemically for the estimation of total soluble salts and organic matter. A pH-meter (WGPYE model 290) was used for the determination of soil pH.

Isolation of keratinophilic fungi from soil samples

The hair baiting technique was employed as recommended by Vanbreuseghem (1952) and as employed by Abdel-Fattah *et al.* (1982): 100 g of soil were put in a sterile plate and a sufficient quantity of sterile distilled water was added and mixed thoroughly to bring about 20-25 % moisture content. Pieces of sterile horse hairs were sprinkled on the surface of moistened soil. Two plates were used for each sample; plates were incubated at 28° C for 6-8 weeks and soil in plates was remoistened whenever necessary. Moulds appearing on the baits were transferred to the surface of Sabouraud dextrose agar medium (Moss & McQuown, 1969) supplemented with 20 unit/ml of sodium penicillin, 40 µ g/ml of dihydrostreptomycin and 0.05 % cycloheximide (Actidione "R"). Before adding to the agar, the first two antibiotics were dissolved separately in sterile distilled water while the third was dissolved in methanol. Plates were incubated at 28° C for 3-4 weeks and developing colonies identified. Frequency of occurrence as percentage of samples and relative important value (RIV) were calculated for each species (Shearer & Webster, 1985; Ali-Shtayah & Asa'd Al-Sheikh, 1988).

Estimation of airborne fungi

Plates (9 cm diam.) containing each 100g soil were moistened with distilled water to about 25-30 %. Horse hair fragments were scattered on the soil surface. Plates were then autoclaved (three times) at 121° C for 30 min. Sterile plate was exposed for 1 h to the atmosphere of swamp; the process was repeated at 50 different sites. Exposed plates were incubated at 28° C for 10-12 weeks and remoistened whenever necessary. Five hair fragments/replicate were then inoculated on plates containing Sabouraud dextrose agar medium, incubated at 28° C for 3-4 weeks and colonies developing from these hair fragments were then identified. Frequency of occurrence as percentage of samples and relative important value (RIV) was calculated for each species (Shearer & Webster, 1985; Ali — Shtayah & Asa'd Al — Sheikh, 1988).

Keratinolytic activity

Sixty-eight isolates of keratinophilic fungi were recovered during the current study; these were used for keratinolysis tests following the English method (1976). Hair-sand cultures were made by scattering 1 cm long pieces of autoclaved hair over the surface of 9 cm Petri dishes containing moist twice-autoclaved sand from the mangrove; plates were inoculated with a 5 ml aqueous spore suspension of each fungus. The hair of fair horse was used in all experiments. After an incubation period of 20 days at room temperature, the amount of fungal growth and sporulation was rated: + for weak growth, ++ for moderate growth, +++ for heavy sporing and spreading cultures.

Test for the antifungal activities of natural products

Twenty fungal isolates of keratinophilic fungi recovered in the present investigation were used to study the antifungal effect of garlic extract and onion oil

Garlic (*Allium sativum* L.) extract

20 g of garlic bulbuls free of scaly leaves were washed several times with sterile distilled water. Bulbuls were homogenised in a sterile blender in 100 ml ethanol (70 %); the mixture was then completed to 200 ml with distilled water to obtain a 10 % garlic extract. The latter was then added to the autoclaved medium (Sabouraud liquid medium) at 3 concentrations: 1000, 2000 and 3000 ppm; a garlic free extract medium was used as control.

Cultures were incubated at 28° C for 15 days.

Onion (*Allium cepa* L.) oil

Onion oil obtained from El-Nasr Company originates from dehydrating agricultural products (A.R.E.). The oil was added to the medium (except for the control) to give concentrations of 100, 200 and 300 ppm. Cultures were incubated at 28° C for 15 days.

RESULTS AND DISCUSSION

Soil samples

The organic matter content and total soluble salts in tested soil samples fluctuated between 3.5-8.4 and 0.5-4.1 %, respectively. All soil samples proved to be alkaline: pH = 7.4-8.5.

Forty-one keratinophilic and cycloheximide resistant species were collected from 50 swamp soil samples baited with horse hair fragments at 28° C.

Aphanoascus (teleomorph of *Chrysosporium*) was the most common genus, occurring in 86 % of the samples and had RIV of 119.7. It was represented by 3 species: *A. fulvescens*, *A. terreus* (anamorph: *Chrysosporium indicum*) and *Aphanoascus* sp. (*Chrysosporium tropicum*). They were present in 60 %, 24 % and 30 % of tested soil samples and developed RIV values of 73.3, 32.4 and 40.5, respectively. *Aphanoascus fulvescens* is known to induce skin infections (Rippon *et al.*, 1970; Albala *et al.*, 1982). The above fungi were previously isolated from soil but with different frequencies in many parts of the world

Genera & species	Soil				Air			
	TI	NCI&OR	RIV	%F	TI	NCI&OR	RIV	%F
<i>Acremonium strictum</i> W. Gams	20	5R	12,8	10	-	-	-	-
<i>Alternaria alternata</i> (Fries) Keissler	12	4R	9,7	8	3	2R	4,2	4
<i>Aphanoascus</i>	240	43H	19,7	86	591	45H	121,3	90
<i>A. fulvescens</i> (Cooke) Apinis	95	30H	73,3	60	211	30H	71,2	60
<i>A. terreus</i> (Randhawa & Sandhu) Apinis	60	12M	32,4	24	180	21M	51,5	42
<i>Aphanoascus</i> sp.	75	15M	40,5	30	195	26M	62,3	52
<i>Apinisia queenslandica</i> Apinis & Rees	3	2R	4,4	4	5	2R	4,3	4
<i>Arthroderma</i>	67	14M	37,4	28	-	-	-	-
<i>A. ciferrii</i> Varsavsky & Ajello	8	2R	5,1	4	-	-	-	-
<i>A. cuniculi</i> Dawson	25	6L	15,5	12	-	-	-	-
<i>A. curreyi</i> Berk	29	7L	18,1	14	-	-	-	-
<i>A. lenticulare</i> Pore, Tsao & Plunkett	5	1R	2,7	2	-	-	-	-
<i>Aspergillus</i>	87	18M	48,2	36	631	47H	127,4	94
<i>A. flavus</i> Link	33	13M	30,6	26	140	36H	79,4	72
<i>A. flavus</i> var. <i>columnaris</i> Raper & Fennell	11	5R	11,5	10	6	2R	4,3	4
<i>A. fumigatus</i> Fresenius	13	5R	11,8	10	160	28H	64,5	56
<i>A. niger</i> Van Tieghem	7	3R	7	6	210	29H	69,1	58
<i>A. sydowii</i> (Bain. & Sart.) Thom & Church	-	-	-	-	53	16M	34,8	32
<i>A. terreus</i> Thom	23	8L	19,2	16	62	10L	23,3	20
<i>Chaetomium globosum</i> Kunze	16	5R	12,2	10	-	-	-	-
<i>Cladosporium</i>	-	-	-	-	45	16M	34,4	32
<i>C. cladosporioides</i> (Fres.) De Vries	-	-	-	-	28	13M	27,5	26
<i>C. sphaerospermum</i> Penzig	-	-	-	-	17	4R	8,9	8
<i>Chrysosporium</i>	105	20M	54,7	40	15	7L	14,8	14
<i>C. asperatum</i> J. W. Carmichael	4	2R	4,6	4	3	1R	2,2	2
<i>C. carmichaelii</i> Van Oorschot	22	5R	3,1	10	-	-	-	-
<i>C. lucknowense</i> Grag	9	2R	5,3	4	-	-	-	-
<i>C. pannicola</i> (Corda) Van Oorschot & Stalpers	12	3R	7,7	6	-	-	-	-
<i>C. pruinatum</i> Gilman & Abbott	3	1R	2,4	2	2	1R	2,1	2
<i>C. xerophilum</i> Pitt	55	14M	35,7	28	10	5R	10,5	10

Genera & species		Soil			Air		
		TI	NCI&OR	RIV	TI	NCI&OR	RIV
<i>Cachilobolus spicifer</i> Nelson	5	3R	6,4	6	110	13M	31,8
<i>Cunninghamella echinulata</i> Thaxter	-	-	-	-	50	16M	34,6
<i>Fusarium oxysporum</i> Shelecht	7	4R	9	III	-	-	-
<i>Gibberella fujikuroi</i> (Sowada) Ito	8	5R	11,1	10	-	-	-
<i>Microrosporum gypsum</i> (Bodin) Guiar & Grigorakis	24	7L	17,4	14	3	1R	2,2
<i>Mucor racemosus</i> Fresenius	8	2R	5,1	4	-	-	-
<i>Myceliophthora vellerea</i> (Sacc. & Spg.) Van Corschor	2	1R	2,3	2	5	2R	4,3
<i>Mycosphaerella tassiana</i> (De Not.) Johanson	-	-	-	120	26H	58,3	52
<i>Nectria haematococca</i> Berk. & Br.	3	3R	6,4	6	-	-	-
<i>Paecilomyces variotii</i> Bain	5	2R	4,7	4	-	-	-
<i>Penicillium</i>	38	5R	15,3	10	251	32H	77,3
<i>P. chrysogenum</i> Thom	8	2R	5,1	4	115	25H	56,1
<i>P. citrinum</i> Thom	6	2R	4,8	4	15	8L	16,8
<i>P. corylophilum</i> Dierckx	-	-	-	28	15M	31,5	30
<i>P. funiculosum</i> Thom	13	5R	11,8	10	63	26H	55,3
<i>P. puberulum</i> Bainier	5	1R	2,7	2	30	10L	21,6
<i>P. variabile</i> Sopp	6	1R	2,8	2	-	-	-
<i>Rhizopus stolonifer</i> (Ehrenb.) Lind	3	2R	4,4	4	-	-	-
<i>Scopulariopsis brevicaulis</i> (Sacc.) Bainier	15	5R	12,1	10	23	13M	27,2
<i>Syncephalastrum racemosum</i> (Cohn.) Schroeter	-	-	-	30	10L	36,5	36
<i>Trichophyton</i>	40	8L	21,6	16	9	3R	6,5
<i>T. ajelloi</i> (Vanbreuseghem) Ajello	6	2R	4,8	4	-	-	-
<i>T. equinum</i> (Mauruchot & Dassonville) Goed.	11	4R	9,5	8	3	1R	2,2
<i>T. mentagrophytes</i> (Robin) Blanchard	18	6L	14,5	12	6	2R	4,3
<i>T. rubrum</i> (Castellani) Sabouraud	5	2R	4,7	4	-	-	-
<i>Verticillium lateritium</i> Berkeley	5	3R	6,7	6	-	-	-
Total isolates	713						
Number of genera	21						
Number of species	41+1 var.						
					1891		
					15		
					29+1 var.		

Table 1 : Total isolates (TI, calculated per 500 hair fragments in all soil samples and per 250 hair fragments in one exposure of 1h), number of cases of isolation (NCI, out of 50 cases), occurrence remarks (OR), relative importance values (RIV) and frequency (% F), calculated per 50 samples) of various fungal genera species recovered from mangrove's soil and air using hair baiting technique at 28° C.

Occurrence remarks : H = high occurrence, isolated from 25 - 50 cases (out of 50) ; M = moderate occurrence, from 13 - 24 cases; L = low occurrence, from 6 - 12 cases; R = rare occurrence, from 1 - 2 cases.

(Piotelli & Caretta, 1974; Mostafa, 1977; Todaro, 1978; Jana *et al.*, 1979; Sur & Ghosh, 1980; Abdel-Fattah *et al.*, 1982; Calvo *et al.*, 1984; Filipello Marchisio, 1986; Chabasse, 1988; Abdel-Hafez *et al.*, 1991; El-Said, 1993).

The anamorphic genus *Chrysosporium* was the second most frequent entity; it was encountered in 40 % of samples analyzed and had RIV of 54.7. For this genus six species were identified with *C. xerophilum* (28 %) being the most common one. The remaining five taxa were rarely recovered; these were *C. asperatum* (4 %), *C. carmichaelii* (10 %), *C. lucknowense* (4 %), *C. pannicola* (6 %) and *C. pruinosum* (2%). All these species were isolated from the soil samples of Oman by El-Said, (1993); there they developed from 6, 12, 10, 10 and 10 %, soil samples respectively. In Egypt, *C. asperatum* and *C. pannicola* were isolated from Egyptian soils by Abdel-Hafez *et al.* (1989, 1991). Filipello Marchisio (1986) isolated *C. pannicola* (3.5 %) and *C. xerophilum* (7.0 %) from children's sandpits in Italy.

Arthroderma occupied the third place with respect to number of cases of isolation of recorded genera; it was recovered from 28 % of samples examined and had RIV of 37.4. Four species were isolated; these are *A. ciferii* (teleomorph of *Chrysosporium georgii*), *A. cuniculi*, *A. curreyi* and *A. lenticulare* (*Trichophyton terrestre*). In Italy, Filipello Marchisio (1986) isolated *A. cuniculi* and *A. curreyi* from children's sandpits. In Oman El-Said (1993) isolated all above *Arthroderma* from soil samples.

Aspergillus (3 species + 1 variety) occupied the fourth place and was encountered in 26 % of the soil samples. Among observed *Aspergilli*, most commonly collected were *A. flavus* and *A. terreus*. The remaining taxa were scarcely recovered; these were *A. flavus* var. *columnaris*, *A. fumigatus* and *A. niger*. Aspergillosis due to *A. fumigatus* and *A. flavus* has a cosmopolitan distribution (Frey *et al.*, 1979). Khalil & Abdel-Sater (1991) isolated *A. flavus*, *A. fumigatus*, *A. niger* and *A. terreus* from mangrove soils of the Egyptian Red Sea Coast. Most of the above *Aspergilli* were previously encountered, but with different incidence from various types of soil from many parts of the world (Sundaram, 1987; Abdel-Hafez *et al.*, 1989; El-Said, 1993, 1994).

Trichophyton developed from 16 % of collected samples. It was represented by *T. tajelloi* and *T. mentagrophytes*. These two species have a wide distribution and are recovered from different substrates (Todaro, 1978; Filipello Marchisio, 1986; Abdel-Hafez *et al.*, 1989; El-Said, 1994). The above two species were found as saprophytes in man and animals, but also have been recognised as causal agents of tinea, onychomycosis and ringworms (Frey *et al.*, 1979).

Microsporium gypseum was observed on only 14 % of the samples. Abdel-Fattah *et al.* (1982) isolated *M. gypseum* from Egyptian soils where it was encountered in 8.5, 2.9 and 7.1 % of soils baited with human hair, animal hair and feathers, respectively. This species is cosmopolitan and it is encountered in different geographic zones (Stepanishcheva, 1965; Belukha & Lukyanova, 1969; Padhyc *et al.*, 1967; Meinhof & Grabowski, 1972; Alilous & Asgar, 1973; Abdel-Hafez *et al.*, 1989). The fungus was reported from skin lesions, feather and pellets of free-living birds, hairs and skin of monkeys, dogs, mice, rats and other small mammals. It was also recognised as the causal agent of dermatomycosis in cattle and in man from different parts of the world (Domsch *et al.*, 1980).

The remaining 13 genera and 15 species recorded were recovered in rare frequencies (Tab. 1).

Airborne fungi

The concentrations and the composition of air-spores trapped at 1.5 m above ground level are shown in Table (1).

Twenty-nine species and 1 variety belonging to 15 genera were recovered from the swamp atmosphere using hair baiting technique at 28° C.

Aphanoascus, a genus closely related to dermatophytes, was common in the air; it emerged in 90 % of exposed samples. *Aphanoascus* also comprised 31.3 % of total fungal catches and had RIV of 121.3. Three species were identified: *A. fulvescens*, *A. terreus* and *Aphanoascus* sp. These occurred in 60 %, 42 % and 52 % of exposures; they comprise 11.2 %, 9.5 % and 10.3 % of total fungi and had RIVs of 71.2, 51.2 and 62.3, respectively. *Aphanoascus terreus* and *Aphanoascus* sp. were recovered previously from the air of Hibis Temple at El-Kharga Oasis in Egypt: there they emerged in 25 % and 33 % of exposures and matched 3.1 % and 16.1 % of total fungi, respectively (Ismail, 1990). Other fungi closely related to dermatophytes were also isolated but with different incidences: *Apinisia queenlandica*, *Chrysosporium asperatum*, *C. pruinatum*, *C. xerophilum*, *Microsporium gypseum*, *Myceliophthora vellerea*, *Trichophyton equinum* and *T. mentagrophytes*. Few numbers of keratinophilic fungi has been encountered previously from the air in other parts of the world (Papavassilion & Bartzokas, 1975; Alteras & Lehrer, 1977; Acosta & Roberstad, 1979; Patil & Kulkarni, 1981; Della-France & Caretta, 1984; Moubasher *et al.*, 1990; El-Maghraby, 1994).

Several moulds were also isolated from the swamp atmosphere using horse hair fragments as bait; these include members of the genera *Alternaria* (1 species), *Aspergillus* (5 + 1 variety), *Cladosporium* (2), *Cochliobolus* (1), *Cunninghamella* (1), *Mycosphaerella* (1), *Penicillium* (5), *Scopulariopsis* (1) and *Syncephalastrum* (1). For these genera most commonly encountered species were: *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *A. sydowii*, *Cladosporium cladosporioides*, *Cochliobolus spicifer*, *Cunninghamella echinulata*, *Mycosphaerella tassiana*, *Penicillium chrysogenum*, *P. corylophilum*, *P. funiculosum*, *Scopulariopsis brevicaulis* and *Syncephalastrum racemosum*. These findings are almost in agreement with those reported by El-Maghraby (1994) during a study on the atmosphere of some schools at Hurghada City; this author also reported that several of the above taxa were then most commonly encountered on goat hair baits lying on Sabouraud dextrose agar. Several of the previous taxa are known to be allergenic (Plutarco, 1958; Masatomo *et al.*, 1991), or causing asthma (Beaumont *et al.*, 1985), able to develop ocular infection (Sehgal *et al.*, 1981), hyper-sensitivity pneumonitis and pulmonary infections (Treger *et al.*, 1985; Arianayagam *et al.*, 1986).

Keratinolytic activity

Table (2) indicate that isolates of different or the same species have variable growth. Most of the isolates (50) showed a reasonable rate (++) of growth. Ten isolates gave higher rates (+++) with abundant vegetative growth. Some isolates have keratin-degrading enzyme(s), but they differ in their capabilities for the production of these enzymes. Peyton *et al.* (1986) recorded a significant keratinolytic activity of *M. canis* and *M. gypseum*. Filippello Marchisio (1986) reported that members of *Microsporium*, *Trichophyton*, *Mariannea*, *Aphanoascus*, *Chrysosporium*, *Malbranchea* and *Geomyces* were the most active keratinolysis. In Egypt, Mahmood (1990) reported that *T. mentagrophytes* was able to grow actively on horse hairs.

Fungal isolates	Rate of growth		
	+	++	+++
<i>Aphanoascus fulvescens</i> (10)	-	5	4
<i>A. terreus</i> (6)	2	4	-
<i>Aphanoascus</i> sp. (8)	-	5	3
<i>Apinisia queenslandica</i> (1)	-	1	-
<i>Arthroderma ciferrii</i> (2)	-	2	-
<i>A. cuniculi</i> (4)	1	3	-
<i>A. curreyi</i> (6)	2	4	-
<i>A. lenticulare</i> (1)	-	1	-
<i>Chrysosporium asperatum</i> (1)	-	1	-
<i>C. carmichaelii</i> (4)	-	4	-
<i>C. lucknowense</i> (1)	-	1	-
<i>C. pannicola</i> (2)	-	2	-
<i>C. pruinatum</i> (1)	-	1	-
<i>C. xerophilum</i> (8)	2	6	-
<i>Microsporium gypseum</i> (5)	-	3	2
<i>Myceliophthora vellerea</i> (1)	-	1	-
<i>Trichophyton ajelloi</i> (1)	-	1	-
<i>T. equinum</i> (2)	1	1	-
<i>T. mentagrophytes</i> (3)	-	2	1
<i>T. rubrum</i> (1)	-	1	-
Total (68)	8	50	10
Percentage (100)	11.8	73.5	14.7

* The number between parentheses indicate the number of isolates tested.+, indicates weak growth; ++, indicates moderate growth; + + +, indicates abundant growth.

Table 2 : growth of fungal isolates on hair- sand medium.

Effect of garlic extract and onion oil ■ isolated keratinophilic fungi.

Garlic extract

All tested fungi were sensitive to garlic extract. Mycelial dry weight of *Aphanoascus fulvescens*, *A. terreus*, *Apinisia queenslandica*, *Arthroderma ciferrii*, *A. cuniculi*, *A. curreyi*, *Chrysosporium asperatum*, *C. carmichaelii*, *C. lucknowense*, *C. pannicola*, *C. pruinatum*, *Microsporium gypseum*, *Trichophyton ajelloi* and *T. mentagrophytes* was significantly reduced by the three tested concentrations. Mycelial dry weight of *Aphanoascus* sp., *Arthroderma lenticulare*, *Chrysosporium xerophilum* and *Trichophyton equinum* significantly depressed by 2000 and 3000 ppm, whereas that of *T. rubrum* was decreased by the 3000 ppm only (Table 3).

Species	Garlic Extract			Onion Oil		
	L (1000ppm)	M (2000ppm)	H (4000ppm)	L 100	M 200	H 400
<i>Aphanoascus fulvescens</i>	60*	43*	0*	75*	33*	15*
<i>A. terreus</i>	74*	52*	12*	82	62*	20*
<i>Aphanoascus</i> sp.	86	74*	68*	92	81*	70*
<i>Apinisia queenlandica</i>	71*	62*	0*	87	63*	22*
<i>Arthroderma ciferrii</i>	53*	42*	0*	66*	54*	0*
<i>A. cuniculi</i>	62*	35*	12*	73*	42*	13*
<i>A. curreyi</i>	50*	0*	0*	65*	41*	0*
<i>A. lenticulare</i>	85	73*	25*	92	87	62*
<i>Chrysosporium asperatum</i>	72*	60*	45*	84	63*	23*
<i>C. carmichaelii</i>	69*	71*	63*	73*	39*	10*
<i>C. lucknowense</i>	76*	53*	25*	86	65*	18*
<i>C. pannicola</i>	68*	32*	0*	75*	52*	0*
<i>C. pruinatum</i>	65*	43*	0*	70*	35*	0*
<i>C. xerophilum</i>	85	70*	35*	90	84	60*
<i>Microsporum gypseum</i>	51*	48*	25*	85	65*	22*
<i>Myceliophthora vellerea</i>	95	84	71*	97	90	85
<i>Trichophyton ajelloi</i>	77*	72*	33*	86	66*	24*
<i>T. equinum</i>	83	75	55	89	72*	65*
<i>T. mentagrophytes</i>	59*	10*	0*	40*	0*	0*
<i>T. rubrum</i>	92	80*	69*	75*	60*	12*

* Means significant difference comparable with the control.

Table 3 : Effect of various concentrations of garlic extract and onion oil on the mycelial dry weight (calculated as percentage of the control) of the test fungi.

Appleton & Tansey (1975) reported that *Epidermophyton floccosum*, *Microsporum canis*, *M. gypseum*, *Trichophyton mentagrophytes*, *T. rubrum* and *Scopulariopsis brevicaulis* do not grow in a concentration of 5.10^{-3} garlic extract. Prasad *et al.* (1982) observed that the topical application of the crude extract of garlic at a 1/10 concentration in distilled water could combat rabbit dermatophytosis induced by *Microsporum canis* without causing any apparent side effects.

Onion oil

The three levels of onion oil inhibited mycelial growth of *Aphanoascus fulvescens*, *Arthroderma ciferrii*, *A. cuniculi*, *A. curreyi*, *Chrysosporium carmichaelii*, *C. pannicola*, *A. purinosum*, *Trichophyton mentagrophytes* and *T. rubrum*. Mycelial growth of *Aphanoascus terreus*, *Aphanoascus* sp., *Apinisia queenlandica*, *Chrysosporium asperatum*, *C. lucknowense*, *Microsporum gypseum*, *Trichophyton ajelloi* and *T. equinum* was significantly retarded by medium and high doses. However, *Arthroderma lenticulare* and *Chrysosporium xerophilum* were significantly retarded by high doses only. On the other hand *Myceliophthora vellerea* was not significantly affected by any level of onion oil (Table 4).

Shekhawat & Prasada (1971) reported that boiled water extracts of onion caused inhibition to growth of *Alternaria tenuis*, *Helminthosporium* sp. and *Curvularia penniseta*. More recently Zohari *et al.* (1992) noticed that onion oil (at 200 ppm) completely inhibited growth of *Microsporum canis*, *M. gypseum* and *Trichophyton simii*, while *Chrysosporium*

queenslandicum and *Trichophyton mentagrophytes* were completely inhibited by a 500 ppm content.

ACKNOWLEDGEMENTS

The author is deeply indebted to Prof Dr. S. I. I. Abdel-Hafez professor of Mycology, Assiut University, Egypt for valuable advices and to Dr. A.M. El-Araby for continuous encouragements.

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